

New Approach to Study Fast and Slow Motions in Lipid Bilayers: Application to Dimyristoylphosphatidylcholine-Cholesterol Interactions

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ABSTRACT Natural abundance ^{13}C solid-state nuclear magnetic resonance spectroscopy was used to investigate the effect of the incorporation of cholesterol on the dynamics of dimyristoylphosphatidylcholine (DMPC) bilayers in the liquid-crystalline phase. In particular, the use of a combination of the cross-polarization and magic angle spinning techniques allows one to obtain very high resolution spectra from which can be distinguished several resonances attributed to the polar head group, the glycerol backbone, and the acyl chains of the lipid molecule. To examine both the fast and slow motions of the lipid bilayers, ^1H spin-lattice relaxation times as well as proton and carbon spin-lattice relaxation times in the rotating frame were measured for each resolved resonance of DMPC. The use of the newly developed ramped-amplitude cross-polarization technique results in a significant increase in the stability of the cross-polarization conditions, especially for molecular groups undergoing rapid motions. The combination of T_1 and $T_{1\rho}$ measurements indicates that the presence of cholesterol significantly decreases the rate and/or amplitude of both the high and low frequency motions in the DMPC bilayers. This effect is particularly important for the lipid acyl chains and the glycerol backbone region.

INTRODUCTION

^1H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy has been widely used to study the structural and dynamic properties of model and biological membranes. In particular, a variety of techniques in ^{13}C natural abundance NMR have been used to study the dynamics of lipid bilayers (De Kruijff, 1978; Cornell, 1980; Brainard and Cordes, 1981; Cornell and Keniry, 1983; Braach-Maksyvtis and Cornell, 1988; Oldfield et al., 1991; Montez et al., 1993). Among them, different relaxation experiments have been established to give a large range of information on the dynamics of the lipids (Gent and Prestegard, 1977; Brown, 1982; Griffin, 1981; Sefcik et al., 1983; Brown et al., 1986). More specifically, spin-lattice relaxation experiments are valuable techniques to obtain information on both the rate and type of motions that occur in bilayers. The spin-lattice relaxation in the laboratory frame (T_1) are sensitive to motions with frequencies in the MHz range (10^8 - 10^{11} Hz). These motions include trans-gauche isomerizations and rotations of molecules around the bilayer normal. In contrast, spin-lattice relaxation in the rotating frame ($T_{1\rho}$) allows the study of slow motions with frequencies in the kHz range. The $T_{1\rho}$ are mainly dominated by shape fluctuations, surface undulations, and slow collective order director fluctuations (Lee et al., 1976; Brown, 1984; Rommel et al., 1988; Bloom et al., 1991; Stohrer et al., 1991). Whereas a substantial amount of experimental information is available on such measurements (Brainard and Cordes, 1981; Cornell et al., 1982; Cornell et al., 1983; de Haan et al., 1985; Ad-

ebodun et al., 1992), most of the existing data are limited to the study of some carbon groups, such as the acyl chain methylenes.

Significant resolution improvement can be achieved by the use of both higher magnetic fields and the magic angle spinning (MAS) technique, which yield very highly resolved spectra (Forbes et al., 1988; Metz et al., 1994). Nevertheless, ^1H MAS spectra exhibit less resolution than that obtained by ^{13}C MAS NMR. Because of the inherent low sensitivity of ^{13}C NMR, the use of the cross-polarization technique (Pines et al., 1973) is of great interest. This method greatly reduces experimental times as the delay between acquisitions depends on the proton spin-lattice relaxation times, which are shorter than those of ^{13}C . In addition, by improving the ^{13}C signal intensity, it allows investigations of membrane systems in ^{13}C natural abundance, i.e., without the need of probe or isotopic labeling. This technique can therefore be of great value for the study of more complex membrane systems.

It has been demonstrated that the determination of the Hartmann-Hahn (HH) condition in cross-polarization (CP) experiments cannot be satisfied for all carbons in complex systems such as biological membranes because the HH matching conditions vary for each carbon of the molecule depending on its local environment. Furthermore, MAS may impair the stability of the CP signal intensity even at low spinning speeds and consequently the reproducibility of relaxation measurements (Peersen et al., 1993). To overcome these problems, a new CP method has been developed by Metz et al. (1994), the so-called ramped-amplitude (RAMP)-CP. This method consists in a gradual increase of the proton or carbon spin-lock field in the rotating frame during the CP time, so that several HH conditions are satisfied. This allows an improvement in the signal-to-noise ratio for some of the carbon resonances and a suppression of HH condition mismatches.

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In the present study, we have investigated the dynamics of dimyristoylphosphatidylcholine (DMPC) bilayers in the hydrated L_α phase, in pure dispersions and in the presence of cholesterol, by relaxation measurement experiments with the combined use of both the MAS and the RAMP-CP techniques. Two types of relaxation experiments have been performed: proton spin-lattice relaxation in the laboratory frame (HT_1) and spin-lattice relaxation in the rotating frame of proton and carbon, named $\text{HT}_{1\rho}$ and $\text{CT}_{1\rho}$, respectively. Because of the high resolution of the spectra, most of the narrow peaks have been resolved and assigned, and relaxation times have been measured for all the resonances of the DMPC molecule. We have thus obtained a very detailed analysis of the motions that occur in the different regions of the lipid molecule, i.e., the polar head group, the glycerol backbone, and the fatty acyl chains, in pure DMPC dispersions as well as in DMPC-cholesterol mixtures. The results clearly show the reliability of these techniques in the study of membrane dynamics.

MATERIALS AND METHODS

Lipid samples

DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine) and 1-myristoyl-2- ^{13}C -myristoyl-*sn*-glycero-3-phosphocholine were supplied by Avanti Polar Lipids (Alabaster, AL), and cholesterol was obtained from Sigma Chemical Co. (St. Louis, MO). The lipids were used without further purification.

DMPC samples were prepared by addition of the appropriate amount of water (50 wt % lipid). DMPC-cholesterol samples were prepared by co-dissolving the appropriate amounts of DMPC and cholesterol (70–30 mol %) in $\text{CHCl}_3/\text{CH}_3\text{OH}$ (2:1 v/v). The solvent was evaporated under a nitrogen stream, followed by high vacuum pumping overnight to remove any trace of solvent. The appropriate amount of water was added (50 wt % lipid) and the samples were kept for 30 min at 50°C , then freeze-thawed and vortexed five times to obtain homogeneous and uniform multilamellar lipid dispersions.

NMR spectroscopy

^{13}C NMR MAS spectra were acquired with a Bruker ASX 300 solid-state NMR spectrometer (Bruker Spectrospin, Milton, Ontario, Canada) operating at a frequency of 75.44 MHz for ^{13}C and a frequency of 300 MHz for ^1H . A broadband/ ^1H dual frequency 4-mm MAS probehead was used for all experiments and was purchased from Bruker Instruments (Bruker Spectrospin).

The spectra were acquired at a temperature of 30°C unless specified otherwise and with a spinning of 2 kHz. The 90° proton pulse length was typically 5.5 μs , and protons were decoupled during data acquisition. The spectra (4096 data points) were acquired with an acquisition time of 0.04 s and a spectral width of 50 kHz. The spectra were zero-filled to 16384 points, and a 10-Hz line broadening was applied to all spectra. The recycle times were 5, 10, and 20 s, respectively, for the HT_1 , the $\text{HT}_{1\rho}$, and the $\text{CT}_{1\rho}$ experiments. The number of scans was 560 for the experiments with pure DMPC dispersions and 720 for the DMPC-cholesterol mixtures. The chemical shifts were referenced relative to external tetramethylsilane. Because long relaxation experiments are particularly sensitive to changes in experimental conditions, short and long τ values were alternated. Furthermore, a minimum of 12 different delay values were used in each experiment. The uncertainty in the T_1 values arising from this procedure is approximately 5%, estimated from replica experiments.

The ^1H spin-lattice relaxation time (HT_1) measurements were carried out with the inversion-recovery method (Maciel et al., 1980). Fig. 1 A shows the pulse sequence used for measuring the ^1H spin-lattice relaxation times in the rotating frame ($\text{HT}_{1\rho}$) in a CP experiment (Stejskal et al., 1981). The

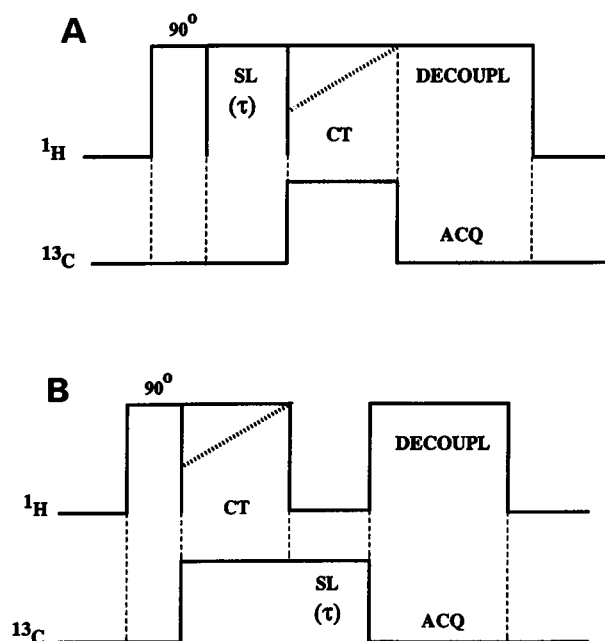


FIGURE 1 Pulse sequences used for the rotating frame relaxation experiments with CP-MAS. (A) $\text{HT}_{1\rho}$. (B) $\text{CT}_{1\rho}$. ACQ, acquisition time; CT, contact time; SL, spin-lock pulse; DECOUPL, decoupling; (τ), variable delay. The diagonal hatched lines show the RAMP-CP.

^{13}C spin-lattice relaxation times in the rotating frame ($\text{CT}_{1\rho}$) were measured as described by Schaefer et al. (1977), and the pulse sequence is shown in Fig. 1 B. However, the conventional CP was replaced by a RAMP-CP in the $\text{HT}_{1\rho}$ and the $\text{CT}_{1\rho}$ experiments. In the conventional CP experiment, the matched spin-lock field strength was 47 kHz. In the RAMP-CP experiments, the CP period began with a 34-kHz proton spin-lock pulse and the amplitude was increased up to 69 kHz. The CP contact times were held at 10 and 15 ms, respectively, for the pure DMPC and the DMPC-cholesterol dispersions in both conventional CP and RAMP-CP experiments. The radiofrequency field amplitude of the spin-lock pulses used in both the $\text{HT}_{1\rho}$ and $\text{CT}_{1\rho}$ experiments was 47 kHz.

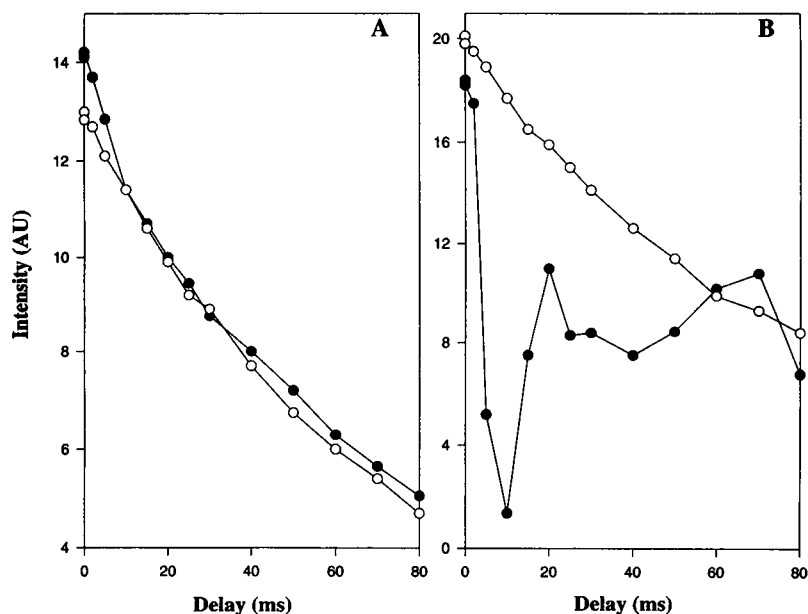
RESULTS AND DISCUSSION

Spectra

Fig. 2 presents the ^{13}C CP MAS NMR spectra of pure DMPC multilamellar dispersions and DMPC-cholesterol mixture (70–30 mol %), in excess water, at 30°C , where the two samples are in the liquid-crystalline lamellar phase. As can be seen from Fig. 2, each sample gives a well resolved ^{13}C NMR spectrum in which there are four main spectral regions: the polar head group, the carbonyl groups, the glycerol backbone, and the aliphatic acyl chains of the DMPC molecule. Some more resonances, belonging to the cholesterol molecule, can also be observed in the spectrum presented in Fig. 2 B.

Most of the carbons in the DMPC molecule can be assigned in the two spectra. The assignments are based on previously reported spectra, and the chemical shift values are very close to those found by Forbes et al. (1988) and Haberkorn et al. (1978). However, it is not possible to assign in the spectrum presented in Fig. 2 B the peaks of the C2 glycerol

FIGURE 3 Magnetization decay of (A) the methylene choline $\text{C}\beta$ and (B) the acyl chain methylenes C4-C11 in pure DMPC multilamellar dispersions as a function of the delay time in the $\text{HT}_{1\rho}$ measurement experiments performed by using conventional CP (\bullet) and RAMP-CP (\circ).



and the methylene carbon $\text{C}\beta$ of the choline head group. Whereas the curves obtained for the C4-C11 carbons are quite similar for both conventional CP and RAMP-CP pulse sequences, it is obvious that the RAMP-CP greatly improves the curve of the $\text{C}\beta$ compared with that obtained with a single-amplitude spin-lock pulse. A similar behavior was also observed for most carbons of the polar head group and of the glycerol backbone region. These results indicate that the groups that undergo rapid motions, such as the polar head group, are significantly affected by MAS even at low spinning speed.

This situation is similar to that observed in adamantane for which the oscillatory matching behavior greatly affects the CP efficiency even at low speed MAS (2 kHz) (Stejskal et al., 1977). Therefore, the use of RAMP-CP can significantly improve the relaxation time measurements in systems in which significant molecular motions occur, like in biological membranes. In addition, the field strength modulation used in RAMP-CP can be beneficial in systems that involve a wide range of different carbon spin systems because of differences in local environments, as is often the case in biological membranes. Furthermore, RAMP-CP improves reproducibility because of the insensitivity of RAMP-CP to exact HH matching conditions (Peersen et al., 1993; Metz et al., 1994).

Relaxation time measurements

The spin-lattice relaxation times in the laboratory frame (T_1) and the spin-lattice relaxation times in the rotating frame ($T_{1\rho}$) are sensitive to different anisotropic motions that occur in phospholipid bilayers. The T_1 are influenced primarily by motions with frequencies close to the Larmor frequency, i.e., motions with frequencies of approximately 10^8 Hz. This means that the high frequency components are expected to dominate the contribution to HT_1 (Jones, 1966; Cornell et al., 1983). In a bilayer, these motions are expected to be rather

restricted in amplitude (Seiter and Chan, 1973; Gent and Prestegard, 1977). In contrast, the $T_{1\rho}$ are more sensitive to the slower motions with frequencies from 10^4 to 10^6 Hz within the bilayer (Sefcik et al., 1983; Cornell et al., 1982). Furthermore, there is a general agreement that the $T_{1\rho}$ are largely spin-lattice in origin even if a small contribution of ^1H - ^{13}C spin-spin relaxation can occur. Therefore, the $T_{1\rho}$ reflect the details of the lipid bilayer molecular dynamics and are sensitive to minor changes in either the motional rate and/or amplitude (Cornell et al., 1983; Sefcik et al., 1983).

Because of the highly resolved nature of the spectra obtained with the CP MAS technique, it is possible to determine the spin-lattice relaxation times of most individual carbon atom sites. We have therefore measured the spin-lattice relaxation times in the laboratory frame (T_1) and the spin-lattice relaxation times in the rotating frame ($T_{1\rho}$) for DMPC bilayers in the absence and presence of cholesterol.

Spin-lattice relaxation times in the laboratory frame (HT_1)

The proton spin-lattice relaxation times in the laboratory frame for the carbon resonances of the pure DMPC multilamellar dispersions and the DMPC-cholesterol mixtures are presented in Table 2 for two different temperatures. The values obtained at 30°C are also illustrated in Fig. 4. In pure DMPC dispersions at 30°C , the HT_1 values of the headgroup and the glycerol backbone are approximately 300 ms with slightly higher values for the $\text{C}\gamma$ carbon of the choline headgroup and the C1 carbon of the glycerol backbone (approximately 350 ms). Higher values are observed for the acyl chain carbons. Moreover, the HT_1 values increase along the chain from the interfacial region to the terminal methyl group. These results show that the motions responsible for the spin-lattice relaxation are more restricted for the glycerol backbone nuclei than for the carbon nuclei in the acyl chain

TABLE 2 HT_1 (in ms) for DMPC and DMPC-cholesterol

		HT ₁ (ms)				
		Type of carbon	DMPC		DMPC-cholesterol	
			30°C	50°C	30°C	40°C
Polar head group	Choline-C γ	344	469	356	280	
	Choline-C β	287	436	328	267	
	Choline-C α	295	432	384	297	
Interfacial region	Glycerol-C3	305	373	491	251	
	Glycerol-C2	295	477			
	Glycerol-C1	362	408	610	315	
Hydrocarbon chains	Chain-C2	401	542	611	488	
	Chain-C3	412	534	633	510	
	Chain-C4-C11	470	624	647	560	
	Chain-C12	556	754			
	Chain-C13	578	794	705	625	
	Chain-C14	647	879	727	636	

and confirm the idea of a motional gradient extending from the glycerol backbone region in both directions as proposed by Brainard and Cordes (1981), Lee et al. (1976), and Godici and Landsberger (1975).

When cholesterol is incorporated into DMPC bilayers, all of the HT_1 values increase at 30°C. This shows that the high frequency motions that contribute to the spin-lattice relaxation are affected by the presence of cholesterol. HT_1 relaxation experiments carried out at higher temperatures (Table 2) allowed us to obtain information about the relation between the HT_1 values and the correlation times of these motions. We have thus found that, in pure DMPC dispersions, the frequencies of the motions contributing to the HT_1 relaxation are higher than the ^1H Larmor frequency (300 MHz), as indicated by the longer HT_1 values obtained with increasing temperature. However, in DMPC-cholesterol mixtures, the HT_1 values decrease with increasing temperature, indicating that the high frequency motions have been slowed down to values below the ^1H Larmor frequency. Therefore, these results indicate that cholesterol causes a great restric-

tion of the high frequency motions, especially those undergone by the glycerol backbone and the acyl chains. Thus, our results show that these regions are more ordered in lipid-cholesterol systems than in pure lipid bilayers in the liquid-crystalline phase. In contrast, the presence of cholesterol seems to affect the polar head group region to a lesser extent.

These observations are in agreement with other ^1H , ^2H , ^{13}C , and ^{19}F NMR studies (Seiter and Chan, 1973; Lancee-Hermkens and de Kruijff, 1977; Oldfield et al., 1978; Davis, 1983; Peng et al., 1989) that have indicated that the presence of cholesterol markedly restricts the local motions of the phospholipid molecules in unsonicated liposomes. In particular, ^2H NMR results have shown that the incorporation of cholesterol causes an increase of the quadrupolar splitting of the fatty acyl chains, which can be explained by a decrease in the extent and rate of trans-gauche isomerization (Siminovich et al., 1988).

Because HT_1 is thought to depend to a large extent on intrachain C-C bond rotation (Petersen and Chan, 1977; Jeffrey et al., 1979), we can conclude that the changes of HT_1 induced by the addition of cholesterol in DMPC bilayers come from a great attenuation of the rotation and kinks in the hydrocarbon chain and glycerol backbone bonds. This conclusion is different from that obtained by Cornell et al. (1982) who had suggested that the presence of cholesterol induces only a small increase in HT_1 relaxation times of the methylene chain protons and concluded that cholesterol has little effect on the fast motions of the acyl chains. However, our results clearly indicate that the high frequency motions of DMPC molecules are greatly restricted by the addition of cholesterol.

Spin-lattice relaxation times in the rotating frame ($T_{1\rho}$)

$HT_{1\rho}$ and $CT_{1\rho}$ of pure DMPC

The spin-lattice relaxation times in the rotating frame of the ^1H and the ^{13}C nuclei (denoted, respectively, $HT_{1\rho}$ and $CT_{1\rho}$) for pure DMPC multilamellar dispersions are given in Tables

FIGURE 4 ^1H spin-lattice relaxation times for the DMPC resonances in pure DMPC (*open bars*) and in DMPC-cholesterol (*hatched bars*) multilamellar dispersions at 30°C. The abscissa represents the different carbon sites as defined in Fig. 2.

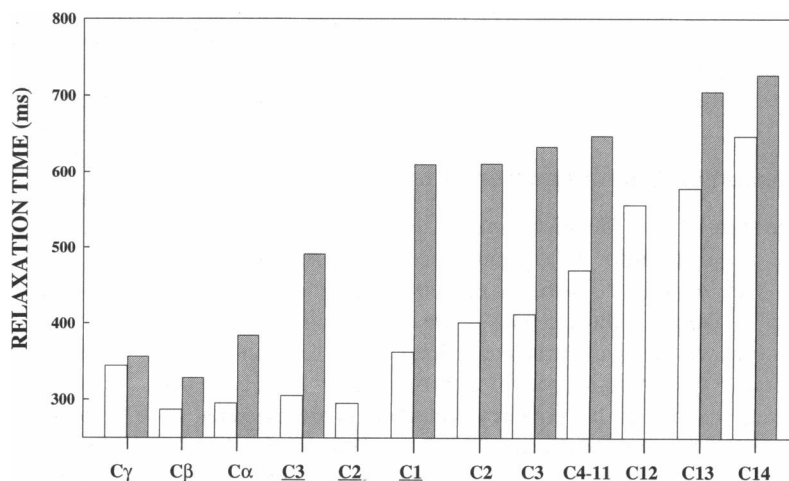


TABLE 3 $\text{HT}_{1\rho}$ (in ms) for DMPC and DMPC-cholesterol

		HT _{1p} (ms)				
		Type of carbon	DMPC		DMPC-cholesterol	
			30°C	50°C	30°C	40°C
Polar head group	Choline-C γ	141	238	106	146	
	Choline-C β	89	153	66	88	
	Choline-C α	86	207	59	92	
Interfacial region	Glycerol-C3	39	75	15	29	
	Glycerol-C2	41	77			
	Glycerol-C1	33	75	15	27	
Hydrocarbon chains	Chain-C2	54	110	22	42	
	Chain-C3	58	123	23	42	
	Chain-C4-C11	64	132	25	46	
	Chain-C12	78	177			
	Chain-C13	95	216	33	51	
	Chain-C14	109	215	35	57	

TABLE 4 $\text{CT}_{1\rho}$ (in ms) for DMPC and DMPC-cholesterol

		CT _{1ρ} (ms)				
		Type of carbon	DMPC		DMPC-cholesterol	
			30°C	50°C	30°C	40°C
Polar head group	Choline-Cγ	361	549	306	337	
	Choline-Cβ	178	302	159	187	
	Choline-Cα	156	266	134	153	
Interfacial region	Glycerol-C3	47	77	24	34	
	Glycerol-C2	62	114			
	Glycerol-C1	43	73	17	28	
Hydrocarbon chains	Chain-C2	72	128	45	62	
	Chain-C3	95	200	107	125	
	Chain-C4-C11	107	223	126	130	
	Chain-C12	158	336			
	Chain-C13	243	624	286	384	
	Chain-C14	679	1270	555	736	

3 and 4 for two different temperatures and are illustrated graphically in Fig. 5 at 30°C. As can be seen in Fig. 5, the $\text{HT}_{1\rho}$ and $\text{CT}_{1\rho}$ values of the DMPC atom sites both have a similar behavior when plotted versus the carbon position. The $\text{CT}_{1\rho}$ values of the glycerol backbone carbons of the DMPC molecules are indeed much smaller than those of the polar head group and the acyl chains. Therefore, the $\text{CT}_{1\rho}$ exhibit a gradient in both directions from the glycerol backbone in a similar way as was observed for the $\text{HT}_{1\rho}$. These results indicate that the slow motions that contribute to the $\text{CT}_{1\rho}$ relaxation are greater in the glycerol backbone region compared with the other regions of the DMPC bilayer. This is consistent with the higher mobility of both the polar head group and the fatty acyl chains.

Effect of cholesterol on the $\text{HT}_{1\rho}$ and $\text{CT}_{1\rho}$ values of DMPC bilayers

The values of $\text{HT}_{1\rho}$ and $\text{CT}_{1\rho}$ of DMPC bilayers in the absence and presence of cholesterol are given respectively in Tables 3 and 4 for two different temperatures, and in Fig. 6 are shown the $\text{HT}_{1\rho}$ and $\text{CT}_{1\rho}$ relative variations ($\text{CT}_{1\rho}(\text{DMPC-}$

cholesterol)- $\text{CT}_{1\rho}(\text{DMPC})/\text{CT}_{1\rho}(\text{DMPC})$) observed for the DMPC atom sites in the presence of cholesterol at 30°C. The experiments performed at higher temperatures indicate that the frequency of the slow motions undergone by the DMPC molecule in both pure lipid dispersions and in DMPC-cholesterol mixtures are higher than the frequency of the B_1 field in the rotating frame, as indicated by the increase in the $\text{CT}_{1\rho}$ values with increasing temperature. The presence of cholesterol in DMPC bilayers is associated with a general decrease in the $\text{HT}_{1\rho}$ values. This decrease is very significant for the fatty acyl chains and the glycerol backbone, indicating an increase of the slow motions contributing to the $\text{HT}_{1\rho}$ relaxation in these regions of DMPC bilayers as a result of the addition of cholesterol. In contrast, the slow motions of the polar head group are much less affected by the incorporation of cholesterol.

The analysis of the $\text{HT}_{1\rho}$ in terms of qualitative molecular dynamics needs a simultaneous comparison with the $\text{HT}_{1\rho}$. The $\text{HT}_{1\rho}$ are mainly influenced by changes in the high frequency motions, whereas $\text{CT}_{1\rho}$ will be influenced by changes in both high frequency and low frequency motions (Cornell

FIGURE 5 ^1H (open bars) and ^{13}C (hatched bars) spin-lattice relaxation times in the rotating frame for the DMPC resonances in pure DMPC multilamellar dispersions at 30°C. The abscissa represents the different carbon sites as defined in Fig. 2.

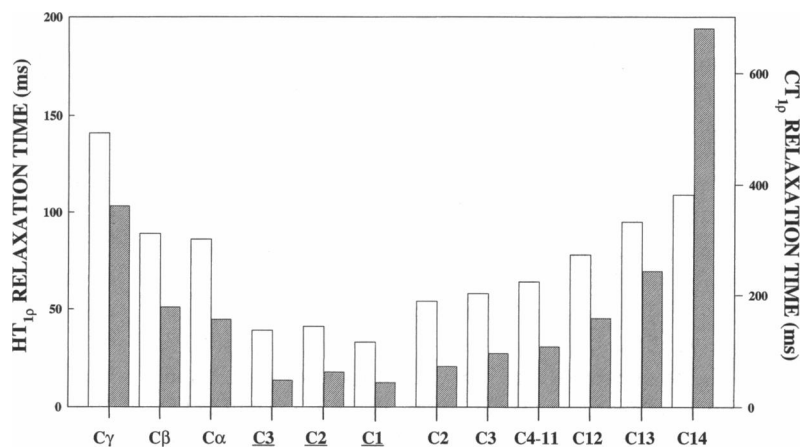
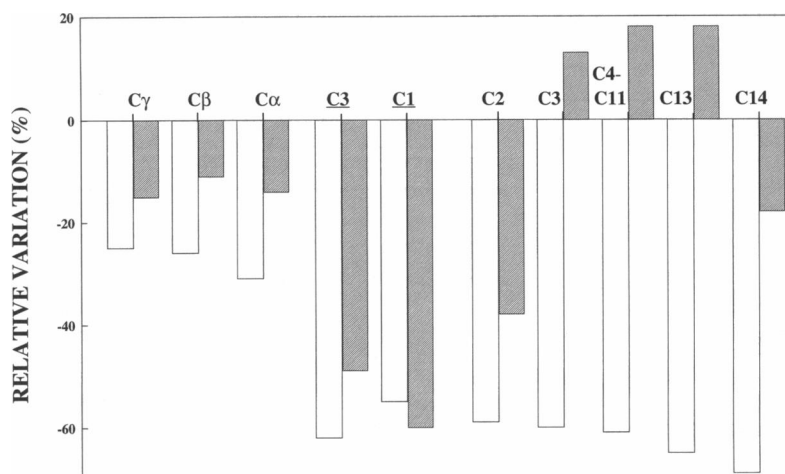


FIGURE 6 Relative variations ($T_{1\rho}$ DMPC-cholesterol- $T_{1\rho}$ DMPC)/ $T_{1\rho}$ DMPC of the $HT_{1\rho}$ (open bars) and the $CT_{1\rho}$ (hatched bars) for the DMPC resonances in DMPC-cholesterol multilamellar dispersions at 30°C. The abscissa represents the different carbon sites as defined in Fig. 2.



et al., 1982; Rommel et al., 1988). Therefore, the changes in $HT_{1\rho}$ could reflect either a decrease of the frequency of the motions near or above 10^8 Hz which could impair the $HT_{1\rho}$ values or an enhancement and/or induction of low frequency motions in the 10^4 - 10^6 Hz range. As discussed earlier, the HT_1 results indicated that the addition of cholesterol in DMPC bilayers results in a significant decrease of the rate of the high frequency motions of both the glycerol backbone and the acyl chain regions. These motions could then contribute to the $HT_{1\rho}$ relaxation processes and result in a decrease in the $HT_{1\rho}$ values. This conclusion differs from that of Cornell et al. (1982), who had suggested that the main effect of cholesterol was to introduce new slow frequency motions in DMPC bilayers and/or to enhance some slow frequency motions that exist at lower intensity in pure DMPC bilayers.

The relative variations of $CT_{1\rho}$ exhibit interesting differences compared with those of $HT_{1\rho}$. First, the effect of cholesterol is slightly less pronounced on the polar head group $CT_{1\rho}$ values than those of the $HT_{1\rho}$. In addition, the $CT_{1\rho}$ of C1 and C3 glycerol are affected to the same extent as the $HT_{1\rho}$. Finally, the influence of cholesterol on the $HT_{1\rho}$ and $CT_{1\rho}$ of the acyl chain carbons is significantly different. Except for the C2 carbon, only small variations in the $CT_{1\rho}$ relative ratios can be observed. These results might be explained by the different motions that influence the $HT_{1\rho}$ and $CT_{1\rho}$. The $HT_{1\rho}$ are influenced by both intramolecular reorientations and fluctuations in intermolecular chain spacing, whereas $CT_{1\rho}$ are mainly dominated by intramolecular C-H dipolar interactions (Cornell et al., 1982; Sefcik et al., 1983; Brown et al., 1986), although rotational rearrangements of the C-H vector can also depend on intermolecular packing. Therefore, the comparison between the $HT_{1\rho}$ and the $CT_{1\rho}$ variations for the different regions of the DMPC molecule suggests that one of the effects of cholesterol is to enhance the fluctuations in the intermolecular acyl chain spacing. On the other hand, the addition of cholesterol increases the frequency of the slow C-H motions of the C1 and C3 carbons of the glycerol backbone as the $CT_{1\rho}$ and $HT_{1\rho}$ values are both dramatically affected. Finally, the changes in

the polar head group $T_{1\rho}$ values are less pronounced, suggesting that the slow motions undergone by this region of the bilayer are less affected by the incorporation of cholesterol. In view of the high resolution achieved in our study, it is clear that the presence of cholesterol does not affect each region of the lipid bilayer to a similar extent. This suggests that local segmental motions in the lipid molecules most likely contribute to the spin-lattice relaxation in the rotating frame. This conclusion does not, however, rule out the contribution of collective motions.

We are also currently investigating in detail the relaxation behavior of the cholesterol resonances. Our results indicate that the values for a specific relaxation time (HT_1 , $HT_{1\rho}$ or $CT_{1\rho}$) are very similar for most of the cholesterol resonances, suggesting that the rotation of the whole cholesterol molecule dominates the relaxation behavior.

CONCLUSIONS

The present study demonstrates that natural abundance ^{13}C solid-state NMR spectroscopy is a valuable technique to investigate the dynamics of lipid bilayers. In particular, we have demonstrated that the use of the RAMP-CP technique greatly improves the stability of the CP condition and therefore allows very accurate measurements of spin-lattice relaxation times, even for groups undergoing very rapid motions. In addition, the very high resolution spectra obtained with the MAS technique allows the simultaneous investigation of the dynamics of the lipid molecules in the polar head group, the glycerol backbone, and the acyl chain regions. Furthermore, as this technique does not require isotopic labeling, it is well suited to investigate the dynamics in more complex systems such as lipid-protein systems and biological membranes. In particular, a detailed knowledge of the dynamics of different regions of the lipid molecules can be very useful to develop editing techniques (Montez et al., 1993) that could lead to considerable simplification of the ^{13}C spectra of complex systems.

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